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The O-2A^{adult} progenitor cell: a glial stem cell of the adult central nervous system

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Systematic comparison of the properties of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells derived from optic nerves of perinatal and adult rats has revealed that these two populations differ in many fundamental properties. In particular, O-2A^{perinatal} progenitor cells are rapidly dividing cells capable of generating large numbers of oligodendrocytes over a relatively short time span. Oligodendrocyte differentiation generally occurs synchronously in all members of a clone, thus leading to elimination of that clone from the pool of dividing cells. However, some O-2A^{perinatal} progenitors are also capable of giving rise to O-2A^{adult} progenitors. These latter cells express many of the characteristics of stem cells of adult animals, including the capacity to undergo asymmetric division and differentiation. We suggest that precursors which function during early development give rise to terminally differentiated end-stage cells and to a second generation of precursors with properties more appropriate for later developmental stages. It is this second generation of precursors which express the properties of stem cells in adult animals, and we therefore further suggest that our work offers novel insights into the possible developmental origin of stem cells.

Key words: progenitor / astrocyte / *perinatal* / *adult*

IN THE LIGHT OF the considerable physiological differences between development and maturity, it has seemed likely that precursor cells which contribute to the early generation of a tissue and the precursors involved in replacement of cells in older animals might differ in their properties. The explosive growth of embryogenesis would be inappropriate in most adult tissues, and processes must exist for slowing down this rapid generation of cells. However, precursor populations cannot be entirely eliminated, as there is a need in adult tissues for the maintenance of a population of precursor cells which would have the capacity to contribute to tissue repair. It is these cells which include the stem cells of adult tissue.

For at least several different cellular lineages, substantial differences have indeed been demonstrated between the precursor cells present during development and in the adult animal. For example, there are fundamental differences between embryonic myoblasts and adult muscle satellite cells,[1,2] and between fetal and adult cells of both the haematopoietic and sympathoadrenal lineages.[3,4] In addition, although optic nerves of both perinatal and adult rats contain progenitor cells which can be induced to differentiate *in vitro* into either oligodendrocytes or type-2 astrocytes,[5-10] the oligodendrocyte-type-2 astrocyte (O-2A) progenitors isolated from optic nerves of adult rats differ from their perinatal counterparts in antigenic phenotype, morphology, cell cycle time, motility and time-course of differentiation *in vitro*.[7-10]

In this review we will discuss our attempts to understand the comparative biology of the precursor cells of developing and mature organisms. These studies have led us to propose functional distinctions between precursors which provide the basis for tissue formation during early development (e.g. neuroepithelial stem cells, embryonic stem cells), but which are not maintained in the animal throughout life, and those which are able to provide a source of new cells in tissues of mature animals. At least for the lineage we have examined, our studies have provided several novel insights into the origin and functional biology of stem cells of adult animals.

The O-2A^{perinatal} progenitor

The first step in our studies on glial development in the CNS was the discovery that cultures derived from white matter tracts of the CNS contained two distinct astrocyte populations, termed type-1 and type-2 astrocytes.[11] These two cell types could be readily distinguished from each other on the basis of morphology, antigenic phenotype and response to growth factors. Most importantly, we found that

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optic nerves of perinatal rats contained a population of glial precursors which did not express glial fibrillary acidic protein (GFAP) at the time of isolation, but which could be induced to become GFAP$^+$ type-2 astrocytes by growth in tissue culture.

Subsequent studies on the precursors of type-2 astrocytes led to the discovery that these cells could also be induced to differentiate into oligodendrocytes.[5] Oligodendrocytic differentiation of O-2A progenitors occured when progenitors were grown in chemically-defined medium and did not require the presence of inducing factors. In contrast, astrocytic differentiation required the presence of appropriate inducing factors, at least one of which is found in fetal sera of a number of different species.

Initial studies on oligodendrocytic differentiation of O-2A progenitors isolated from optic nerves of perinatal rats presented the paradox that the cells we were studying were isolated at a time of maximal division of this lineage *in vivo*,[12,13] yet cells did not divide in tissue culture. Resolution of this paradox began with the discovery that cortical astrocytes promoted O-2A^{perinatal} progenitor division *in vitro*.[14] The astrocytes used in these studies express many of the properties of type-1 astrocytes of the optic nerve, which are the first identifiable glial cells to appear in the nerve.[15] The similarity of these two populations led us to suggest that type-1 astrocytes were responsible for supplying the mitogen(s) required to keep O-2A progenitors in division *in vivo*. Moreover, populations of O-2A^{perinatal} progenitors grown in the presence of purified cortical astrocytes were capable of undergoing extended division while also continuing to generate more oligodendrocytes,[14] a pattern of behaviour similar to that occurring *in vivo*. Thus, the failure of O-2A^{perinatal} progenitors to divide in our initial *in vitro* studies was due to the lack of necessary mitogens, which appeared to be supplied by another glial cell type of the optic nerve.

Further studies demonstrated that purified cortical astrocytes could also promote the correctly timed differentiation *in vitro* of O-2A^{perinatal} progenitors isolated from optic nerves of embryonic rats.[16] The molecular mechanism by which this timing is controlled remains a mystery, although all evidence to date indicates that it is the O-2A^{perinatal} progenitors themselves which are measuring elapsed time.[17,18] A potential linkage between the measurement of elapsed time by dividing cells and the control of differentiation has also been observed for fibroblasts and haematopoietic stem cells (for review see ref 19). In the case of O-2A^{perinatal} progenitors, it appears that this biological clock causes clonally related dividing progenitors to differentiate synchronously into oligodendrocytes within a limited number of cell divisions.[10,17,18] However, it is not yet known whether the mechanism which underlies this synchronous differentiation of clones of dividing cells is also responsible for the first appearance of oligodendrocytes in the rat optic nerve at the day of birth *in vivo*, or the equivalent time *in vitro*.

The effects of purified cortical astrocytes, and of type-1 astrocytes from the optic nerve, on O-2A^{perinatal} progenitor division *in vitro* appear to be mediated by platelet-derived growth factor (PDGF).[18,20-22] O-2A^{perinatal} progenitors exposed to either PDGF or astrocyte-conditioned medium exhibited a bipolar morphology, migrated extensively (with average migration rates of $24.6 \pm 5.6\ \mu m\ h^{-1}$) and divided with an average cell cycle length of 20 ± 6 h. PDGF was also as potent as type-1 astrocytes at promoting the correctly timed differentiation *in vitro* of embryonic O-2A progenitors into oligodendrocytes.[18] Moreover, antibodies to PDGF blocked the mitogenic effect of type-1 astrocytes on embryonic O-2A progenitor cells, causing these cells to cease division and to differentiate prematurely even when growing on monolayers of type-1 astrocytes. Thus, this single mitogen was able to elicit a complex behavioural phenotype from O-2A^{perinatal} progenitors which included normal functioning of the cellular mechanisms involved in the measurement of elapsed time. Interestingly, recent studies have indicated that neurons, which also promote division of O-2A^{perinatal} progenitors *in vitro*,[22,23] may also be a source of PDGF.[24,25] However, the specific contributions of either neuronal or astrocytic[26] production of PDGF to the development of the O-2A lineage *in vivo* is not yet known.

O-A^{adult} progenitors

To attempt to gain insights into the cellular mechanisms underlying regeneration of the oligodendrocyte population following demyelinating damage *in vivo*, we also initiated studies on O-2A progenitors of the adult CNS. In our initial studies, which again were focused on the rat optic nerve, we found that O-2A progenitors isolated from adult animals differed from their perinatal counterparts in several ways. When co-cultured with purified cortical astrocytes, O-2A^{adult} progenitors had a unipolar morphology *in vitro*,[7] whereas O-2A^{perinatal} progenitors were usually bipolar.[7,27] In

addition, O-2A^{adult} progenitors had a longer average cell cycle time *in vitro* than O-2A^{perinatal} progenitors (65 ± 18 h versus 18 ± 4 h),[7,20] migrated more slowly ($4.3 \pm 0.7\ \mu m\ h^{-1}$ versus $21.4 \pm 1.6\ \mu m\ h^{-1}$),[7,27] and take longer to differentiate (3-5 days versus 2 days for 50% differentiation).[7] Furthermore, O-2A^{adult} progenitors stimulated to divide by purified cortical astrocytes were O4$^+$ while dividing O-2A^{perinatal} progenitors were O4$^-$ (ref 7; I. Sommer, M. Noble, unpublished observations).

The appearance of adult-specific precursors in any lineage raises questions about their developmental origin. Are these cells derived from a common ancestor cell which, for example, initially gives rise to O-2A^{perinatal} progenitors, and then gives rise to O-2A^{adult} progenitors during later stages of development? Alternatively, are perinatal and adult precursor populations derived from two distinct ancestors, despite being specialized to produce similar terminally differentiated end-stage cells?

The continued presence of precursor populations in adult animals also raises questions about how such populations are maintained within any particular tissue throughout life. The maintenance of a precursor population throughout life is generally thought to be associated with the presence of a stem cell population which supplies new cells to the precursor pool for use in cell replacement following normal turnover or injury. For example, it has been suggested that the presence of proliferating O-2A progenitors in the adult animal requires the existence of a pre-progenitor, or stem cell, compartment in the O-2A lineage.[6] The requirement for a stem cell compartment to support the prolonged maintenance of dividing O-2A progenitors in the nerve is further indicated by the self-extinguishing nature of the O-2A^{perinatal} progenitor population. As described earlier, O-2A^{perinatal} progenitors grown *in vitro* in the presence of purified cortical astrocytes (as a source of PDGF)[14,20] generally divide and differentiate symmetrically, such that all members of a clonal family of cells synchronously differentiate into oligodendrocytes within a limited number of divisions.[17,18] It is clear that this mode of division and differentiation is incompatible with continued self-renewal of precursors throughout life, and it was thus not surprising to find that O-2A^{perinatal} progenitors are present only in small numbers in cultures prepared from optic nerves of 1-month-old rats[8] and are not detectable in cultures prepared from optic nerves of adult rats.[7]

Generation of O-2A^{adult} progenitors from O-2A^{perinatal} progenitors

Analysis of the development of O-2A^{adult} progenitor cells in cultures derived from 3-week-old rats, the age when the relative proportion of perinatal to adult O-2A progenitors appears to be changing most rapidly *in vivo*,[8] has indicated that some O-2A^{perinatal} progenitor-like cells have the ability to generate O-2A^{adult} progenitor-like cells when co-cultured with purified cortical astrocytes.[10] These experiments were carried out by filming the behaviour of families of cells derived from single O-2A progenitors. Due to the simplicity of the optic nerve cultures, and our extensive characterization of the cell types found in these cultures, the morphological information provided in these films could be used to identify with great precision O-2A progenitors, and to distinguish between cells with *perinatal*- or *adult*-like phenotypes.

In seven individual time-lapse microcinematographic films, with a total analysis of 15 separate families of O-2A lineage cells, we found four examples of families in which (1) the founder cell gave rise at the first division to two cells with the characteristic morphology, cell-cycle length and motility of O-2A^{perinatal} progenitors and (2) subsequent to the first division, members of the family expressed the unipolar morphology, lengthened division times and slow migration rates typical of O-2A^{adult} progenitors.

Figure 1 depicts diagramatically the history of one of the families wherein O-2A^{perinatal} progenitor-like cells were seen to give rise to O-2A^{adult} progenitor-like cells. In this family the founder cell first generated two further O-2A^{perinatal} progenitor-like cells (cells a and b). The family branch represented by one of these progenitors (cell a) terminated, over two divisions, with the production of three oligodendrocytes (cells c, d and e, which were characterized by their multipolar morphology, lack of division and lack of migratory behaviour).[20,31] The other branch (cell b) first produced three further perinatal progenitor-like cells before all of these cells started to express longer cell cycle times and migration rates. By the next division, all of the motile and dividing members of this family expressed a unipolar morphology, a cell cycle length of >40 h ($x = 45$ h) and a migration rate of $\leqslant 6\ \mu m\ h^{-1}$ ($x = 4\ \mu m\ h^{-1}$); see cells f, g and h in Figure 1. Similar observations were made in the other three families in which a *perinatal*-to-*adult*-transition was observed.[10]

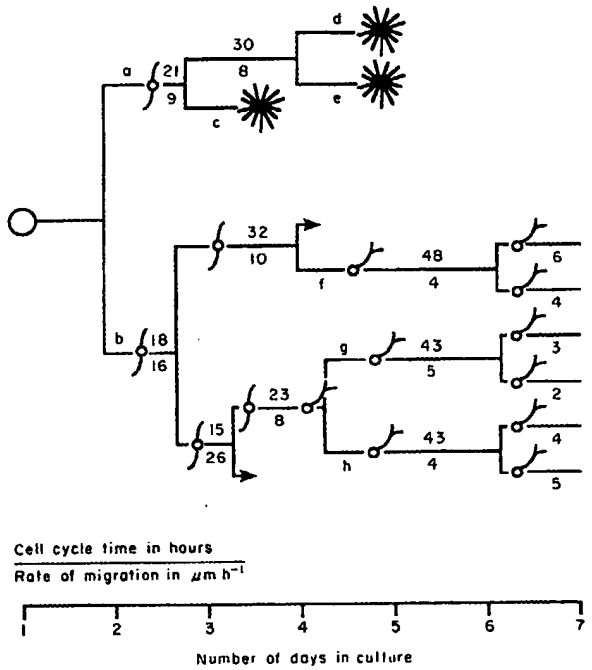

Figure 1. Bipolar O-2A^{perinatal} progenitor-like cells that divide and migrate at a fast rate give rise to unipolar O-2A^{adult} progenitor-like cells which migrate and divide more slowly. Fifteen clonal colonies of O-2A progenitors, stimulated to divide by cortical astrocyte-derived mitogens, were followed by time-lapse microcinematography. Within 15 colonies suitable for detailed analysis, four clear examples were found of O-2A^{perinatal} progenitor-like cells that were bipolar, were highly motile and had a short cell cycle time, which in the first division generated more O-2A^{perinatal} progenitor-like cells, and which eventually gave rise to cells which expressed the unipolar morphology, lengthened cell cycle time and slow migration rate of O-2A^{adult} progenitors. One of the families in which dividing O-2A^{perinatal} progenitor gave rise to O-2A^{adult} progenitor-like cells is represented diagramatically in the figure. The morphology of a progeny cell is indicated in the figure only when the cell was clearly bipolar, unipolar, or oligodendrocyte-like. Since some progeny cells moved out of the field of photography (depicted with an arrow) their fate could not be determined. The numbers above the lines are the cell cycle times in hours, while the numbers below the lines are the migration rates in $\mu m\ h^{-1}$. The transitions shown could not be ascribed to changes in the composition of the tissue culture medium, since all still cultures contained actively dividing and migrating O-2A^{perinatal} progenitor-like cells at the end of the filming period.

Our time-lapse observations suggest that the transition from *perinatal* to *adult* phenotype is not an abrupt one, in that generation of O-2A^{adult} progenitor-like cells may require two or more cell divisions, with the cells present after one division expressing cell-cycle times and motility characteristics intermediate between the *perinatal* and *adult* phenotypes. These results also are consistent with our previous studies on the characteristics of cells derived from optic nerves of 1-week to 1-month-old rats, in which we observed cells with phenotypes which could not be classified unambiguously as *adult*-like or *perinatal*-like.[8]

Extended self-renewal in the O-2A lineage is associated with the *in vitro* generation of O-2A^{adult} progenitors

To test further the hypothesis that O-2A^{adult} progenitors might be derived from O-2A^{perinatal} progenitors, we then serially passaged perinatal optic nerve cells over the course of 3 months. In these experiments, optic nerve populations containing O-2A^{perinatal} progenitors, but not O-2A^{adult} progenitors, were passaged onto fresh monolayers of purified and irradiated cortical astrocytes for up to six passages.

Serial passaging of O-2A progenitors derived from optic nerves of perinatal rats was associated with a shift in the progenitor population from entirely *perinatal*-like to predominantly *adult*-like, as judged by antigenic and morphological criteria and by changes in the population doubling times.[10] In the early passage cultures, dividing O-2A progenitor-like cells (identified by [^{3}H]- thymidine labelling, immunolabelling and autoradiography) expressed the bipolar morphology and A2B5$^+$O4$^-$ antigenic phenotype characteristic of O-2A^{perinatal} progenitor cells. In contrast, >80% of the dividing O-2A progenitors in the later passage cultures expressed the O4$^+$ antigenic phenotype characteristic of O-2A^{adult} progenitors, and 92% of these cells also expressed the characteristic unipolar morphology of O-2A^{adult} progenitors.[7] The rate of increase in the numbers of new progenitors and oligodendrocytes in these cultures also decreased significantly with increasing passage number, and fell from the 24 h doubling times characteristic of perinatal populations to approach the long doubling times characteristic of adult populations. In agreement with the increase in the average doubling time with increasing passage number, the proportion of progenitor-like cells which incorporated [^{3}H]-thymidine during a 24 h pulse decreased with successive passages.

The results of our serial passaging experiments were thus consistent with the hypothesis that O-2A^{adult} progenitors are derived from O-2A^{perinatal}

progenitors and further suggested that expression of the capacity for prolonged self-renewal in this lineage is associated with the appearance of O-2A^{adult} progenitors. The mechanism which might underly such self-renewal was suggested by observations that serial passaging was associated with a slight increase in the proportion of colonies which contained both oligodendrocytes and [^{3}H]-thymidine labelled O-2A progenitors, an observation examined in closer detail using cells derived from adult animals (see next section).

Characteristics of division and differentiation in colonies of O-2A^{adult} progenitor cells

As O-2A^{perinatal} progenitors are not detected at all in cultures derived from adult optic nerves, it is unlikely that generation of O-2A^{adult} progenitors from O-2A^{perinatal} progenitors is the mechanism which allows maintenance of the *adult* progenitor in the nerve throughout life. The slight increase in the proportion of O-2A lineage colonies containing both oligodendrocytes and [^{3}H]-thymidine labelled O-2A progenitors, seen in our passaging experiments, raised the possibility that O-2A^{adult} progenitors might be able to divide and differentiate asymmetrically. Such a pattern of division and differentiation would allow these cells to give rise to more progenitors and generate oligodendrocytes at a slow rate. To examine this possibility under conditions which would allow cells to undergo several divisions, we analysed the composition and size of oligodendrocyte-containing colonies generated from O-2A^{perinatal} and O-2A^{adult} progenitors grown at clonal densities (<1 cell/30 mm^2) on monolayers of purified cortical astrocytes (to promote progenitor division).[7,14] As the generation of O-2A^{adult} progenitors from O-2A^{perinatal} progenitors (as would occur in cultures derived from 3-week-old rats) would have complicated analysis of these experiments, the optic nerve cells used in these experiments were obtained exclusively from newborn and adult rats. Colonies were examined after a length of time which would allow cells to undergo $\leqslant 6$ divisions and $\leqslant 10$ divisions, this being 6 and 10 days for O-2A^{perinatal} progenitors and 15 and 25 days for O-2A^{adult} progenitors, respectively.

Oligodendrocyte-containing colonies

As in previous experiments,[17] the composition and size of oligodendrocyte-containing colonies derived from O-2A^{perinatal} progenitors were consistent with the view that the generation of oligodendrocytes by these cells is associated with symmetric division and clonal differentiation. Sixty-six percent of the oligodendrocyte-containing colonies examined on Day 10 consisted entirely of oligodendrocytes and, even as early as Day 6, the A2B5$^+$GalC$^-$ progenitor-like cells in mixed colonies were most frequently multipolar non-dividing cells (i.e. not labelled with [^{3}H]-thymidine) which appeared to have been visualized just prior to oligodendrocytic differentiation. Only 7% of the oligodendrocyte-containing colonies derived from *perinatal* progenitors and visualized on Day 6, and 14% of those visualized on Day 10, contained both oligodendrocytes and dividing progenitor cells (as judged by the incorporation of [^{3}H]-thymidine). Moreover, oligodendrocyte-containing colonies derived from O-2A^{perinatal} progenitors clustered around sizes of 2, 4, 8, 16, 32, 64 and 128 cells/colony at 6, 8 and 10 days after plating, as expected when clonally-related cells divide symmetrically and differentiate synchronously.

Unlike the results obtained with O-2A^{perinatal} progenitors, the composition of oligodendrocyte-containing colonies derived from individual dividing O-2A^{adult} progenitors was consistent with the hypothesis that the generation of oligodendrocytes by these cells occurred by means of asymmetric division and differentiation.[10] Over 75% of the oligodendrocyte-containing colonies derived from individual O-2A^{adult} progenitors grown at clonal densities contained both oligodendrocytes (which generally do not divide in these tissue culture conditions)[7,14] and [^{3}H]-thymidine-labelled progenitors after both 15 and 25 days of *in vitro* growth, periods of time which would allow $\leqslant 6$ or $\leqslant 10$ average cell cycles for O-2A^{adult} progenitors.[7] The proportion of colonies which contained both oligodendrocytes and radiolabelled O-2A progenitor cells on Days 15 and 25 of *in vitro* growth was very similar, even though the average size of the oligodendrocyte-containing colonies continued to increase with time in culture (from a median value of 7 cells/colony on Day 15 to a median value of 11 cells/colony on Day 25). Only 10% of the colonies visualized on Day 25 consisted entirely of oligodendrocytes, and the remaining 14% contained oligodendrocytes and progenitors which were unlabelled by [^{3}H]-thymidine. In addition sizes of oligodendrocyte-containing colonies did not cluster at factors of 2 on either Day 15 or Day 25 of *in vitro* growth.

Oligodendrocyte-free colonies

Sixty-two percent (110/176) of the colonies derived from O-2A^{adult} progenitors contained no oligodendrocytes even after 25 days of *in vitro* growth. Oligodendrocyte-free colonies seen at this stage were generally small, and over 80% of these colonies (89/110) contained $\leqslant 16$ cells at Day 25. Consistent with the small size of many of these colonies, $<20\%$ (23/110) of the oligodendrocyte-free colonies contained any cells wich were labelled by a 20 h pulse with [^{3}H]-thymidine. In contrast, in colonies derived from O-2A^{perinatal} progenitors, only 30% (41/136) of the colonies were free of oligodendrocytes on Day 10 *in vitro*.

The O-2A^{adult} progenitor as a stem cell

While the generation of O-2A^{adult} progenitors from O-2A^{perinatal} progenitors provides a possible explanation for the origin of the *adult* cell, the lack of O-2A^{perinatal} progenitors in adult optic nerve[7] suggests that other mechanisms are involved in maintenance of a dividing population of O-2A^{adult} progenitor in the adult animal. Although it has been previously suggested[6] that the presence of such cells in the adult requires the existence of an ancestral stem cell, capable of generating O-2A lineage cells throughout life, several observations now raise the possibility that the O-2A^{adult} progenitors may themselves function as stem cells.

The first stem-cell like property of O-2A^{adult} progenitors derives from the observation that this population is maintained in the rat optic nerve as a dividing population seemingly throughout life (ref 6; G. Wolswijk, E. Abney, unpublished observations). O-2A^{adult} progenitor-like cells can be isolated from optic nerves during the first week after birth and such cells remain in the nerve for at least the first year of life, in contrast with O-2A^{perinatal} progenitors, which have largely disappeared from the optic nerve by one month after birth.[7,8] *In vitro* observations suggest that O-2A^{perinatal} progenitors would disappear from the nerve as a consequence of symmetric differentiation of most clones of cells into oligodendrocytes and differentiation of the remaining cells into O-2A^{adult} progenitors (and possibly type-2 astrocytes, although the *in vivo* existence of these cells is controversial; see refs 28-32).

The second stem-cell like property of O-2A^{adult} progenitors is their long (60-65 h) cell cycle times.[7-9] Our most recent studies [10] further suggest that the population of O-2A^{adult} progenitors may even contain a sizeable proportion of cells with cell cycle times in excess of 100 h. Examination of colonies developing *in vitro* over 25 days showed that the great majority (89/110) of these colonies contained $\leqslant 16$ cells after 25 days *in vitro*, and that only a small proportion (23/110) of these colonies contained cells which were labelled with a 20 h pulse of [^{3}H]-thymidine. Both of these results are consistent with the existence of O-2A^{adult} progenitors with very long cell-cycle times.

Also of potential relevance to the question of whether O-2A^{adult} progenitors express stem-cell like characteristics are our observations consistent with the view that these cells can undergo asymmetric division and differentiation *in vitro*. Unlike colonies derived from O-2A^{perinatal} progenitors, oligodendrocyte-containing colonies derived from O-2A^{adult} progenitors generally contained O-2A^{adult} progenitors which were labelled by [^{3}H]-thymidine, indicating that onset of differentiation in the *adult* progenitor-derived colonies was not associated with cessation of cell division in the whole colony. The capacity to undergo asymmetric division and differentiation is an important attribute of *bona fide* stem cells of adult animals.

A further stem-cell like feature displayed by *adult* progenitors grown *in vitro* was that a far higher proportion of oligodendrocyte-containing colonies than oligodendrocyte-free colonies contained O-2A^{adult} progenitors labelled with a 20 h pulse of [^{3}H]-thymidine (75 versus 20%; ref 10). Similarly, the onset of differentiation of epidermal stem cells into keratinocytes in any clone of cells is associated with an increased likelihood of finding cells engaged in DNA synthesis, in association with passage of stem cell progeny through a transit amplifying population of cells engaged in differentiation.[33]

Growth factor co-operation and self-renewal in the O-2A lineage

All of the research described thus far was carried out in cultures in which O-2A progenitor division was promoted either by purified cortical astrocytes or by PDGF (the progenitor mitogen secreted by these cells). However, we have also found that there are other developmental programmes which can be expressed by dividing O-2A progenitors. As will be discussed below, some of these findings may be of particular relevance to understanding the control of

precursor self-renewal and also to the elicitation from adult stem cells of a pattern of growth likely to be of importance in responding to tissue injury.

O-2A^{perinatal} progenitors division can be induced by exposure to cells to basic fibroblast growth factor (bFGF), but cells induced to divide by this mitogen were multipolar and showed little migratory behaviour.[34] In addition, cells induced to divide by bFGF had a cell-cycle length of 45 ± 12 h, in contrast with the 18 ± 4 h cell cycle length elicited by exposure to PDGF. These results indicate that PDGF and bFGF function in the O-2A lineage as modulators of differentiation as well as functioning as promoters of cell division. PDGF and bFGF also differ in their effects on oligodendrocytes themselves, in that only bFGF is able to promote division of these cells.[34-36]

The effect of bFGF on oligodendrocytic differentiation of O-2A^{perinatal} progenitors is currently a subject of controversy. In our initial studies, we found that O-2A progenitors exposed to bFGF differentiated prematurely to form oligodendrocytes.[34] In contrast, other investigators found that this same mitogen inhibited differentiation of purified O-2A^{perinatal} progenitors.[37] The several methodological differences between the two sets of studies (ranging from the source of progenitors to the methods of tissue culture) make it difficult to determine the reasons for these differing observations. Our more recent studies do however suggest that at least part of the discrepancy between the two sets of results may have been düe to effects of other factors present in the cultures, and that bFGF does indeed inhibit oligodendrocytic differentiation of purified O-2A^{perinatal} progenitors.[46]

In respect to O-2A ^{perinatal} progenitors, the most intriguing results of our studies with PDGF and bFGF was the discovery that progenitors exposed simultaneously to these two mitogens continued to divide without differentiating into oligodendrocytes.[34] For example, cultures prepared from optic nerves of 19-day-old rat embryos began to generate oligodendrocytes after 2 days when established in the presence of PDGF alone,[18,34] yet remained oligodendrocyte-free even after 10 days of growth in the presence of PDGF + bFGF.[34] Further experimentation has demonstrated that O-2A^{perinatal} progenitors can be grown continually for a year or more *in vitro* as long as cells are continually exposed to both of these mitogens (S.C. Barnett, M. Noble, unpublished observations). O-2A^{perinatal} progenitors grown in this manner retain the ability to undergo oligodendrocytic differentiation when removed from the presence of both mitogens. We do not yet know whether O-2A^{perinatal} progenitors grown for extended periods in this manner will generate O-2A^{adult} progenitors.

The discovery that cooperation between growth factors can cause prolonged self-renewal of precursors revealed a previously unkown means of regulating self-renewal in a precursor population. Such cooperation may, however, represent a more general phenomenon, as indicated by the importance of growth factor cooperation in promoting the extended division *in vitro* of haematopoietic stem cells[38] and primordial germ cells.[47] It will be of considerable interest to determine the extent to which cooperation between different growth factors is responsible for eliciting particular aspects of stem cell behaviour.

Growth factor cooperativity and lesion repair

While it is difficult to determine the role (if any) played by PDGF/bFGF cooperativity during development, some of our most recent studies have suggested that such cooperativity may be of profound importance in the context of lesion repair. These studies have also revealed a further property of O-2A^{adult} progenitors of relevance in considering the stem cell-like behaviour of these cells.

We have recently found that simultaneous exposure of O-2A^{adult} progenitors to PDGF + bFGF converts many of these cells to a rapidly dividing and highly motile phenotype with a bipolar morphology and antigenic phenotype very similar to that expressed by O-2A^{perinatal} progenitors.[48] Thus, these cells can be induced to express a phenotype which seems likely to be of relevance to repair of demyelating lesions. These findings demonstrate that the molecular mechanisms which underlie the characteristic behaviour of O-2A^{perinatal} progenitors are not irreversibly inactivated with the generation of O-2A^{adult} progenitors, but are instead placed under the control of slightly different signalling processes than those which function in the perinatal cells. The finding that rapid cell division can be induced in O-2A^{adult} progenitors is consistent with observations that repair of virally-induced demyelination *in vivo* appears to be preceded by increases in the numbers of O-2A^{adult} progenitor-like cells.[39] In addition, studies in other laboratories have suggested an increased production of FGFs and PDGF following CNS damage.[40-42] It is particularly intriguing, however, that our studies also suggest that the ability of O-2A^{adult} progenitors to maintain a

rapidly dividing and migrating phenotype is not maintained beyond a small number of divisions, suggesting intrinsic limitations may exist in the extent to which these cells are capable of contributing to myelin repair.[48] Such a possibility is reminiscent of claims that MS lesions are initially repaired, but eventually become permanently demyelinated.

A revised view of the O-2A lineage

Figure 2 summarizes some of our current views about development of the O-2A lineage, in which the

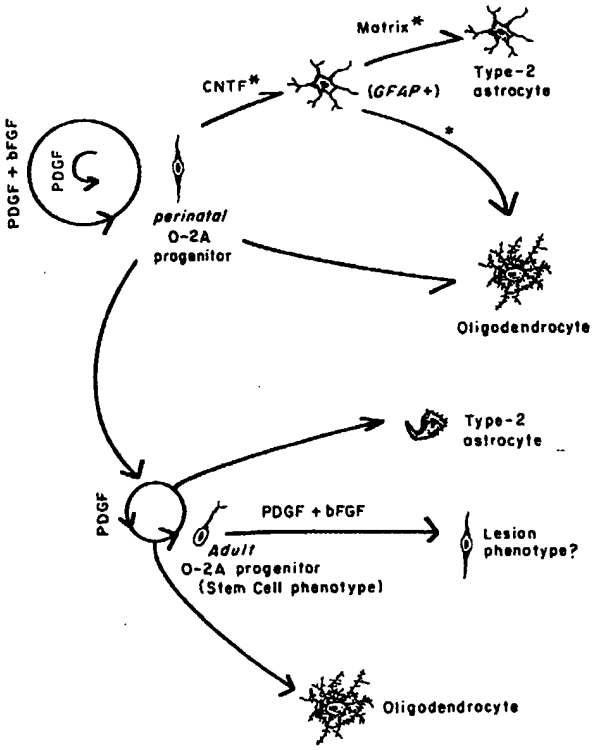

Figure 2. The O-2A lineage, as presently perceived in our laboratory. *Ciliary neurotrophic factor (CNTF) is thought to initiate astrocytic differentiation of O-2A progenitors, as detected by expression of glial fibrillary acidic protein (GFAP). Full differentiation of O-2A^{perinatal} progenitors into type-2 astrocytes, however, requires the additional presence of an unidentified extracellular matrix component (see refs 43, 44). Differentiation of O-2A^{adult} progenitors into type-2 astrocytes has not yet been extensively studied, although we have observed that the type-2 astrocytes generated following growth in serum-containing medium do not express the stellate morphology of type-2 astrocytes derived from O-2A^{perinatal} progenitors.[7] For more detailed discussion on other aspects of differentiation in this lineage see also refs 21, 29, 45.

population of O-2A^{perinatal} progenitors is now seen as tripotential and capable of giving rise to oligodendrocytes, type-2 astrocytes and O-2A^{adult} progenitors. Our studies suggest that O-2A^{perinatal} progenitors express the properties of true progenitor cells, in that these cells generally express a limited life-span before undergoing differentiation (at least when stimulated by purified cortical astrocytes or PDGF). However, a previously unanticipated differentiation pathway which appears to be open to O-2A^{perinatal} progenitors is to give rise to a new generation of precursors, these being the stem cell-like O-2A^{adult} progenitors. The apparent development of O-2A^{adult} progenitors, with stem cell-like characteristics, from a rapidly dividing perinatal population differs significantly from the pattern of development seen in other lineages, where slowly dividing stem cells (of developed, rather than developing, tissues) have been seen to give rise to rapidly dividing progenitors (for review see ref 33). However, no other studies have focused on the origin of potential stem populations in the manner in which we have.

A general hypothesis on the origin of stem cells, which we believe emerges from our studies, is as follows: precursors which function during early development express properties required for cells participating in the initial creation of a tissue. Such properties are inappropriate at later developmental stages, at least for some tissues. In such instances, the early precursors give rise to a second generation of precursors with properties more appropriate to later development, as well as to terminally differentiated end-stage cells. It is this second generation of precursors which represent the stem cells of adult animals. Unfortunately, it will first be necessary to be able to distinguish unambiguously between fetal (or perinatal) and adult precursor cells in other tissues before it can be determined whether phenomena similar to those observed in the O-2A lineage also occur in other lineages.

In regards to the O-2A lineage itself, there are many challenging questions which remain unanswered. It will first be important to determine whether the process we have described for the O-2A lineage of the optic nerve occurs in all regions of the CNS. On a more fundamental level, it will be necessary to determine the relationship between the symmetric and asymmetric O-2A^{perinatal} progenitors. Are these two cell types distinct from the beginning of their existence, is one cell type the ancestor of the other, or do they represent two possible differentiation pathways of a still earlier ancestor cell? Moreover,

although it seems likely that it is the asymmetrically behaving O-2A^{perinatal} progenitors which eventually give rise to 0-2A^{adult} progenitors, the mechanism which causes the earlier cells to generate cells of an *adult* phenotype is a mystery. At present, we know that O-2A^{perinatal} and O-2A^{adult} progenitors exposed to platelet-derived growth factor (PDGF) each express their characteristic morphologies, migratory properties and cell cycle lengths.[9,20] It will be an important challenge to define the molecular alterations which allow a single cell-signalling molecule to elicit such different behaviours from these two precursor populations, and to determine whether these alterations are alone sufficient to convert O-2A^{perinatal} progenitors into O-2A^{adult} progenitors. It is also interesting that this replacement of an O-2A^{perinatal} progenitor population by an O-2A^{adult} progenitor population *in vitro* is at least superficially similar to that which occurs *in vivo* (albeit over a slightly shorter time-scale than that seen *in vitro*). The ability to reproduce such a conversion in tissue culture will facilitate future studies on molecular mechanisms which might be involved in the generation of O-2A^{adult} progenitors from O-2A^{perinatal} progenitors. Finally, the O-2A^{adult} progenitor cell may offer a suitable model system for probing the molecular mechanisms involved in the generation of asymmetric division and differentiation.

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